Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat and Meat Products - MLG 5C.04

SCOPE

This method is applicable for detection and isolation of Top 7 Shiga-toxin producing *E. coli* (STEC) (*E. coli* O157, O26, O45, O103, O111, O121 and O145) in meat products.

PRINCIPLES

MLG 5C.04 utilises GENE-UP PCR detection assays, GENE-UP® STEC - *stx* & *eae* (EH1) and GENE-UP® Pathogenic *E. coli* (PEC), in the screening followed by cultural isolation. EH1 PCR detects the *stx* and *eae* genes in a sample, while PEC PCR detects the presence of *stx* and *eae* genes in the same cell. Positive samples then undergo serogroup specific PCR analysis (GENE-UP STEC Top 6 and O157:H7 PCR). Cultural isolation of the screen positive sample requires the use of immuno-concentration using VIDAS® *E. coli* serogroups (ESPT), followed by plating on modified Rainbow Agar (mRBA). Colonies are then tested for specific O antigens using latex agglutination and PCR. Positive colonies are purified on Sheep Blood Agar (SBA) for confirmation. Colonies on SBA are tested for the presence of O antigens, then identified using the Bruker® MALDI Biotyper and a confirmation rapid screening PCR.

The detection of STEC can be broken down into the following steps:

- **Enrichment**
  Samples (325 ± 32.5 g) are diluted in 975 ± 19.5 mL mTSB. If meat pieces are overweight, prepare a second sub-sample that must be ≥ 63 and ≤ 357.5 g at 1:4 dilution. Samples and diluent are stomached and incubated static at 42 ± 1°C for 15-24 h. A positive control must be included. It is recommended that a negative control (*E. coli* ATCC 25922) and a blank are also run with each batch of samples.

  Note: DAFF export sample weight collected is 375 ± 37.5 g. Therefore, when using this method, a sub-sample needs to be prepared and analysed.

- **Rapid Screening PCR for *stx*/*eae* and O-group**
  Samples are screened for the presence of *stx* and *eae* using EH1 and PEC PCR assays following the GENE-UP User's Guide. Samples negative for *stx* and *eae* targets are considered negative for Top 7 STEC. Samples that are positive for *stx* and *eae* genes but negative for PEC PCR are considered negative. Samples that test positive on the PEC PCR will be further analysed by GENE-UP STEC Top 6 and GENE-UP O157:H7 PCR to determine if one of the Top 7 serogroups is present. Samples negative for these serogroups are considered negative for Top 7 STEC. A positive result for *stx*/*eae* and O group is regarded as potential positive.

- **Isolation and Identification**
  Immuno-concentration and streaking onto mRBA
  All potential positive samples from PCR screening are immuno-concentrated on the VIDAS® *E. coli* serogroups (ESPT) kit by using the ESP2 Protocol followed by plating onto mRBA. All plates are incubated at 35 ± 2 °C for 20-24 h.

  Examination of mRBA
  E. coli O157:H7 colonies typically have black or grey colouration. For all six non-O157, see MLG 5C Appendix 2 for colony characteristics.

  Presumptive Rapid Screening
  Colonies are picked from all plates and tested for agglutination with O antiserum. At least one colony of each morphological type on each plate is tested using latex and *stx*/*eae* PCR assay. Samples that have no growth on mRBA or colonies that are agglutination/PCR negative are reported as negative for STEC. Agglutination/PCR positive samples are regarded as presumptive positive.

- **Confirmation**
  Presumptive positive colonies are to be steaked on SBA (incubate at 35 ± 2°C for 16 – 24 h) for confirmation including a positive control of interest. Check for contamination. Perform proteomic confirmatory tests using Bruker MALDI Biotyper or validated equivalent system (or biochemical[i] tests, see end note next page). Perform serological identification using latex agglutination. Agglutination sample must be further analysed for *stx* and *eae* genes by PCR and serogroup PCR. A sample that confirms as an *E. coli* isolate containing *stx* and *eae* genes, and one of the target serogroups is regarded as confirmed positive STEC.

CHECKLIST

Enrichment	Is the sample enriched in mTSB?	
	Is enrichment carried out at 42 ± 1 °C for 15-24 h?	
	Is the correct amount of enrichment broth used (i.e. 975 ±19.5 mL of mTSB for 325 ± 32.5 g sample)?	
	Is a sub-sample processed and enriched at 1:4 dilution (i.e. one portion of meat in three portions of broth)?	
	Are a positive control and a blank run with each batch of samples analysed?	
	Are control cultures inoculated into enrichment broth at a level of 10 to 100 cells?	
Screening	Is screening for *stx* and *eae* undertaken using EH1 and PEC PCR assays?	
	Is analysis for serogroup specific genes carried out using GENE-UP STEC Top 6 and O157:H7 PCR assays?	
	Is a cocktail of Top 7 STEC cultures run with each PCR?	
Immuno-concentration	Is immuno-concentration performed using the VIDAS *E. coli* Serogroups (ESPT) kit?	
	Is the ESP2 Protocol used for immuno-concentration?	
Isolation	Are filtrates and dilutions from immuno-concentrate used to steak on mRBA?	
	Are all plates incubated at 35 ± 2 °C for 20-24 h?	
	Are all morphological types from all plates confirmed by latex agglutination and then PCR?	
Confirmation	Are latex and PCR positive colonies streaked onto SBA and incubated at 35 ± 2 °C for 16-24 h?	
	Are colonies on SBA analysed for *E. coli* using a Bruker MALDI Biotyper or a validated equivalent system?	
	Are a portion of colonies on SBA confirmed by latex agglutination?	
	Are latex positive colonies confirmed for *stx*/*eae* by PCR assays?	
	Are latex positive colonies confirmed for serogroups using serogroup specific PCR?	

[i]Biochemical Confirmation
A laboratory may elect to use biochemical confirmation methods (VITEK® 2) if a Bruker® MALDI Biotyper is unavailable or there is an interruption in the reagent supply chain. Inoculate isolate onto VITEK® 2 GN cards (if using VITEK® 2 Compact) or equivalent. An isolate is positive on VITEK® 2 if biochemically identified as *E. coli.*